

Induction of Callus from *Crataegus cuneata* Stems

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The fruits of *Crataegus cuneata* are used as a diuretic and peptic agent. And also the fruits are used as medicine for intestinal disorders. Callus was induced from stem of *C. cuneata*. High frequency of callus formation was obtain with the medium containing more than 10 μ M of NAA.

Introduction

The fruits of *Crataegus cuneata* Sieb. are used as a diuretic and peptic agent. And also the fruits are used as medicine for intestinal disorders.

In this paper, we report the induction of callus from stems of *C. cuneata*.

Materials and Methods

Induction and culture of callus

A herb garden grown *C. cuneata* plants of approximately ten years of age were used (Fig. 1.). Stems of this plant were rinsed in 70% (v/v) ethanol for 30 seconds, sterilized by immersion for 5 minutes in 5% sodium hypochlorite solution containing 0.01ml/L of Tween 80, and rinsed three times in sterilized distilled water. The sterilized stems were cut into explants (20mm x 5mm) with a surgical knife and placed on Murashige and Skoog medium (MS medium)¹⁾ supplemented with 3% sucrose, vari-

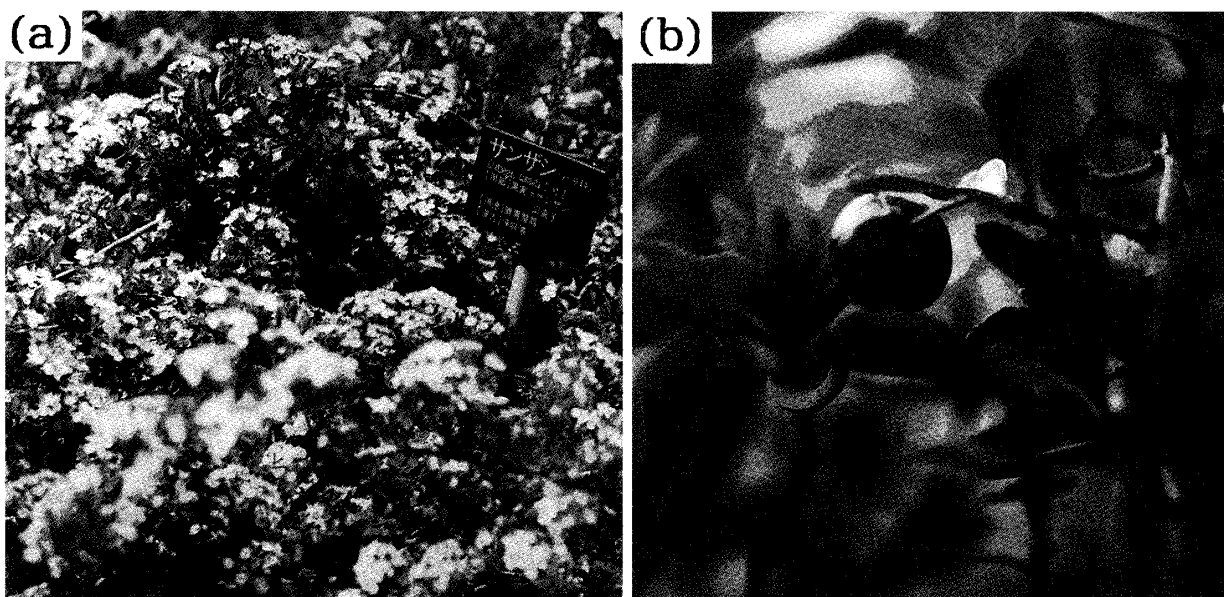


Fig. 1. *C. cuneata*((a), (b)).

ous concentration (0, 0.01, 0.1, 1, 10 μ M) of 1-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), 6-benzyladenine (BA) and kinetin. The solid medium was solidified by 0.2% Gelrite. The culture were incubated in dark at 25°C.

Results and Discussion

Induction and culture of callus

Callus formation from stem explants was observed within 17–36 days of culture as shown in Table 1. The media containing NAA and BA effectively induced callus. Typical callus was formed in the test tube with the medium containing 0.1 μ M of IAA and 0.1 μ M of BA (a), and that contained 10 μ M of NAA and 10 μ M of BA (b) as shown in Fig. 2.

Table 1. Effects of NAA, IAA, BA and kinetin on callus formation in *C. cuneata* stem.

Hormone	Callus formation (%)	
	17 days	36 days
NAA:KI*	33	42
NAA:BA	45	55
IAA:KI	18	40
IAA:BA	32	43

*KI, Kinetin

Table 2. Effects of concentration of NAA and BA on callus formation in *C. cuneata* stem.

Concentration of NAA (μ M)	Concentration of BA (μ M)	Callus formation (%)
0	0	0
0	0.01	0
0	0.1	65
0	1	35
0	10	0
0.01	0	0
0.01	0.01	0
0.01	0.1	65
0.01	1	100
0.01	10	0
0.1	0	0
0.1	0.01	100
0.1	0.1	100
0.1	1	100
0.1	10	0
1	0	65
1	0.01	100
1	0.1	100
1	1	100
1	10	65
10	0	100
10	0.01	100
10	0.1	100
10	1	100
10	10	100

Callus was observed after 42 days culture.

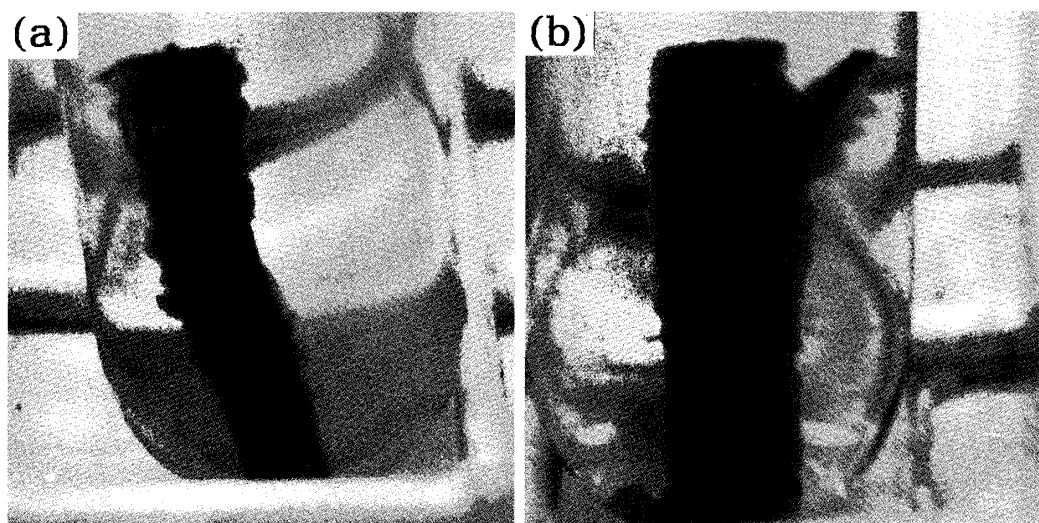


Fig. 2. Callus formation from a peeled stem of *C. cuneata*.

(a) The medium contained 0.1 μ M of IAA and 0.1 μ M of BA.

(b) The medium contained 10 μ M of NAA and 10 μ M of BA.

Table 2. shows the frequency of embryogenic callus formation from stem explants after 42 days culture. In particular, the better results were obtained with the medium containing $0.01\mu\text{M}$ of NAA and $1\mu\text{M}$ of BA. High frequency of callus formation was obtained with the medium containing more than $10\mu\text{M}$ of NAA.

Acknowledgments

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References

- 1) Murashige, T. and Skoog, F., *Physiol. Plant.*, **15**, 473-497 (1962).